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Phenotypes of peripheral blood lymphoid cells in patients with asbestos-related pleural lesions

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Phenotypes of peripheral blood lymphoid cells in patients with asbestos-related-pleural lesions. M. Özesmi, G. Hillerdal, A. Karlsson-Parra, U. Forsum.

ABSTRACT: Asbestos-related parietal pleural plaques develop slowly and are of little clinical significance. Other asbestos-related pleural reactions, for example acute exudative pleurisy and progressive pleural fibrosis, are of clinical importance. The pathogenesis of these reactions is unknown, but one hypothesis is that immunological disturbances are involved. To investigate this hypothesis a phenotypic characterization of lymphoid cells was performed in the peripheral blood of 45 patients with asbestos-related pleural lesions; 20 with pleural plaques (PP), 15 with diffuse pleural fibrosis (DPF), and 10 with benign asbestos pleural effusion (BAPE). Twenty-four healthy blood donors were used as controls. All asbestos groups together had a significantly higher percentage of B-cells than the controls. The percentage number of "helper/inducer" T-cells was significantly lower in the BAPE and DPF groups than in the control and PP groups. Thus, significant aberrations in peripheral blood lymphoid cells were found in patients with DPF and BAPE. This differed from patients with PP only who were similar to normals.

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Exposure to asbestos can cause both benign reactions and malignant tumours in man [1] and can also affect the immune system [2]. Pleural changes are common, and most commonly seen are parietal pleural plaques (PP). These consist of dense collagen tissue and typically show a gradual development without any sudden progression [3]. The lung itself is not affected by the plaques, although a parenchymal fibrosis may be present concomitantly.

Two other asbestos-related reactions of the pleura are benign asbestos pleural effusion (BAPE) and diffuse pleural fibrosis (DPF). BAPE typically appears suddenly, tends to reappear after drainage for some months, but finally resolves, sometimes leaving DPF [4]. DPF can result from a BAPE, but it can also occur without any apparent such episode. DPF can be progressive. The thickening is usually at its greatest at the costophrenic angle which is almost invariably rounded. Typical of this type of lesion is that the thickening seems to involve mainly the visceral pleura, and the lung parenchyma is always involved to some extent [5, 6]. The difference between DPF and PP is best appreciated in the computer scan, where PP are always clearly demarcated from an underlying healthy lung while in DPF patients the peripheral lung parenchyma is involved and there are fibrous strands reaching deep into the lung [5]. NAVRATIL and DOBIAS [6] gave the first extensive description of these pleural changes and named them "hyalinosis complicata", while the plaques were called "hyalinosis simplex". The erythrocyte sedimentation rate (ESR) is significantly more often elevated in patients with DPF than in those with pleural plaques alone, indicating some type of on-going inflammatory activity in the former [7].

The present study was undertaken to investigate whether there were any disturbances in the immune system of persons with various asbestos-related pleural changes.

Material and methods

Patients

At the Department of Lung Medicine in Uppsala, Sweden, a large number of persons with asbestos-related pleural and parenchymal changes are seen [3]. They were divided into three groups according to the findings on their chest roentgenograms:

1. Pleural plaques only (PP), the criteria being bilateral plaques at least 5 mm thick [3],
2. Benign asbestos pleural effusion (BAPE), where tuberculosis, malignancy and other possible causes of the effusion were excluded [4],
3. Bilateral diffuse pleural thickening of various degrees (DPF), with rounded costophrenic angles but without pleural effusion.

Consecutive patients, all male, were collected. The number of patients with PP was limited to 20. There were 15 patients with DPF and 10 with BAPE. They were all occupationally exposed to asbestos, mainly as building workers, and most had had only intermittent exposure. They had been exposed for a mean of 20 years, with extremes of 3 to 35 years. There was no difference in the intensity or length of exposure between the various groups (PP, BAPE and DPF).

Controls

Twenty-four healthy blood donors were used as controls.

Peripheral blood mononuclear cells

Mononuclear cells in heparinized venous blood were isolated by standard methods in a Ficoll-Hypaque density gradient. The cells were then washed three times in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) at 10^6 cells·ml^{-1}. Approximately 10 µl of the cell suspension was dropped into each well of a multiple well microscope slide. The slides were air-dried at +37°C for one hour and then stored at -70°C. After storage the cell preparations were immediately fixed in acetone diluted 1:1 with water (+4°C) for 1 min and finally fixed in 100% acetone (+4°C) for 5 min before staining [8].

Antisera and other reagents

The murine monoclonals denoted anti-Leu 4, -Leu 2a, -Leu 3a, -Leu 7, -Leu 11b, -Leu 10, -Leu 12 and anti-HLA-DR, were all obtained from Becton Dickinson (Sunnyvale, California, USA). Anti-Leu 4 defines all peripheral T-cells [9], whereas anti-Leu 2a defines the "suppressor/cytotoxic" T-cell subset [10]. Anti-Leu 3a react with the "helper/inducer" T-cell subset [10], but also reacts with some cells of the monocyte/macrophage lineage [11] in prefixed cell samples. Anti-Leu 12 defines all peripheral B-cells [12]. Anti-Leu 10 is specific for the HLA-DQ antigen, a human D region associated antigen [13], which differs from the HLA-DR antigen recognized by the anti-HLA-DR monoclonal antibody [14]. The human D region associated antigens are expressed in B lymphocytes, monocytes/macrophages and activated T-cells. The anti-Leu 7 and anti-Leu 11b monoclonals are thought to define the major natural killer (NK) and killer (K) cell subsets [15,16].

FITC-conjugated goat anti-mouse antibody was obtained from the National Bacteriological Laboratory (SBL), Sweden. Biotinylated horse anti-mouse IgG, avidin DH and biotinylated horseradish peroxidase H ("ABC" kit) were obtained from Vector laboratories (Burlingame, California, USA). 3-Amino-9-ethylcarbazole was obtained from Sigma (St Louis, Mo, USA).

Immunoenzyme staining

After the fixation procedure the slides were washed in PBC (pH 7.4) for 5 min. The subsequent incubations were carried out sequentially at room temperature and the slides were washed for 5 min in the PBS buffer between each step. Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 for 15 min. The cell preparations were then incubated for 30 min in a humid atmosphere with 25 µl portions of monoclonal antibodies (dilution 1/32 for anti-Leu 4, -Leu 2a, -Leu 3a, -Leu 10 and -Leu 12, and 1/128 for anti-HLA-DR in PBS containing 4% BSA). Biotinylated horse-antimouse IgG (dilution 1/400) was allowed to react with the cell preparation for 30 min. A complex of 5 µl of avidin DH (10 mg·ml^{-1}) and of 5 µl of biotinylated horseradish peroxidase H (5 mg·ml^{-1}) in 400 ul of PBS was then layered on the cell preparation for 30 min. The peroxidase reaction was developed with the use of a carbazole-containing buffer for 15 min. The cell preparations were exposed to haematoxylin for nuclear staining and mounted in Kaiser's glycerin-gelatin (Merck, Darmstadt). The percentage of positive cells with a margin membrane staining pattern [9] was determined by counting 200 to 500 mononuclear cells in a routine light microscope.

Immunofluorescence staining

When using first-step monoclonals of IgM class (anti-Leu 7 and anti-Leu 11b antibodies), FITC-conjugated goat anti-mouse Ig was used as the second-step reagent. The cell preparations were incubated for 30 min with 25 µl portions of monoclonal antibodies (dilution 1/80 for Leu 7 and 1/20 for Leu 11b in PBS containing 4 % BSA), washed for 5 min in PBS and then incubated with FITC anti-mouse immunoglobulin (dilution 1/320 in PBS containing 4 % BSA) for 30 min. After being washed in PBS for 10 min, the cell preparations were mounted in PBS-glycerine. The cells were examined under a Leitz epifluorescence microscope. The percentage of positive cells was determined by counting 200 cells. The primary antibody was omitted for the negative controls.

Statistical methods

Student's t test (two-tails) was used.

Results

There were no significant difference between the various asbestos groups regarding age, but the normal controls were a little younger. The mean values for ESR and for various subsets of lymphocytes are given in table 1.

Table 1. – Age, erythrocyte sedimentation rate (ESR) and various lymphocyte subsets (within bracket deviations)

	Units	Normals (control)	Pleural plaques (PP)	Diffuse pleural fibrosis (DPF)	Asbestos effusion (BAPE)
n		24	20	15	10
Age	yrs	46.8	65.9	61.7	62.4
		(15.0)	(16.7)	(19.6)	(24.2)
ESR	$mm \cdot h^{-1}$		14.4	20.2*	31.6***
			(19.40)	(26.0)	(34.9)
Leu 10 (HLA-DQ)	%	11.5	11.4	13.7	13.3
		(7.6)	(10.2)	(17.2)	(12.3)
Leu 4 (all T-cells)	%	63.0	56.8*	50.1***	59.4
		(11.4)	(16.2)	(19.3)	(13.1)
Leu 2a(suppr/cytotox)	%	24.1	27.5	23.5	26.4
		(9.0)	(17.8)	(18.7)	(13.1)
Leu 3a(helper/inducer)	%	42.9	41.7	32.8***	33.3***
		(12.2)	(18.5)	(22.0)	(17.2)
Leu 12 (B-cells)	%	4.2	5.2	4.8	6.3
		(4.2)	(8.2)	(4.1)	(5.6)

* ** ***; Statistically significant from normals at the 5, 1, and 0.5 per cent level, respectively

Lymphoid cell phenotypes. B-cells

All asbestos groups (PP, DPF and BAPE) combined had a significantly higher percentage of B cells than the normal controls ($p<0.01$).

T-cells

The mean percentage of T-cells (Leu 4) was lower in the PP and, particularly, in the DPF group than in the normal controls ($p<0.05$ and $p<0.005$, respectively), but there was no significant difference between the BAPE group and control group. The mean value for the PP group was lower than that for the BAPE group but not significantly so, and lower than the DPF group ($p<0.05$); the mean value for the DPF group was significantly lower than that for the BAPE group ($p<0.005$).

For the "helper/inducer" (Leu 3a) T-cells, groups DPF and BAPE both had a significantly lower value than the controls ($p<0.005$). The PP group, however, was not significantly different from the control group, but there was a significant difference between PP and the combined DPF and BAPE groups ($p<0.01$), PP having a higher value. No significant difference was found for the "suppressor/cytotoxic" (Leu 2a) T-cells.

There were no significant differences between the groups regarding the Leu 10+ (HLA-DQ), HLA-DR+, Leu 12+ cells (the B cells) or Leu 7+ and Leu 11b+ (NK and K cells subsets).

The changes in Leu 3a and the non-significant changes in Leu 2a subsets followed each other to some extent, and hence there were no differences between the various groups for the Leu 3a/Leu 2a ratio. In normal controls, this ratio was 1.89; in the PP group 1.66; in the BAPE group 1.52; and in the DPF group 1.66. For all three asbestos groups combined, the figure was 1.63, which was not statistically different from that of the normal controls.

ESR: The erythrocyte sedimentation rate differed significantly between the PP and the BAPE group ($p<0.005$) and between the PP group and the combined group of BAPE and DPF ($p<0.005$).

Correlations: ESR correlated with changes in the percentage numbers of B cell (Leu 12+) in the group BAPE ($r=0.51$) and in groups DPF and BAPE combined ($r=0.49$), but not in group DPF alone, nor in group PP ($r=-0.03$). There were no correlations between any other lymphocyte subsets and ESR in any group.

Discussion

In persons exposed to asbestos several immunological derangements have been demonstrated [2]. The most consistent findings are in increase in the B-cell lymphocyte activity, with increased levels of various immunoglobulins and auto-antibodies, and defective T-cell activity, manifested by weak or absent delayed cutaneous reactions to common antigens such as tuberculin protein [17]. A decrease in T lymphocytes in the blood has been reported [18–20] and both subsets ("helper/inducer" [18] and "suppressor/cytotoxic" [18, 21, 22]) seem to be affected.

The number of B lymphocytes has been reported to be both decreased [23] and increased [21] in the blood of persons exposed to asbestos, but the consistent reports of elevated levels of immunoglobulins [2, 19, 24] and auto-antibodies such as rheumatoid and anti-nuclear

factors [2, 25–27] indicate that these cells must be hyperactive. This hyperactivity might be due to a direct effect of asbestos on B-cells and/or defective suppressor T-cell function. In the present study the B-cells were slightly increased in number in the combined asbestos group.

More interesting than the changes in the combined group of asbestos-exposed persons is the fact that significant differences in the lymphocyte subsets were observed between groups with clinically and radiologically different asbestos-related changes. In particular, the PP group differed in many aspects to a greater extent from the BAPE/DPF group than from the normals, as seen in table 1. This was especially true for the "helper/inducer" T-cells. These findings are in accordance with the clinical findings of NAVRATIL and DOBIAS, [6] who divided the pleural changes into "hyalinosis simplex" and "complicata".

A hypothesis which fits with these data is that in some individuals exposed to asbestos only minor immunological changes will develop; these persons will show pleural plaques only. Other persons, however, are prone to a more severe immunological derangement; these are ones who develop BAPE and/or DPF. One manifestation of DPF is the development of rounded atelectasis [5]. Patients with such lesions have earlier been shown to have immunological abnormalities, which fit well with our findings [28].

Since an intact immunoregulatory network is thought to be of importance for protection against tumours, the observed numerical imbalance between different lymphocyte subsets might have further consequences. DPF and BAPE seem to be fairly common in populations with a high risk of malignant mesothelioma but rare in other asbestos-exposed populations where this risk is low [29,30]. The question of whether the immunological imbalance observed here is of any aetiological importance for the development of malignant tumours or represent only a secondary epiphenomena necessitates further studies.

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Les Phénotypiques des cellules lymphoïdes périphériques de patients atteints de lésion pleurale d'origine asbestosique.
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RÉSUMÉ: Les plaques pleurales pariétales d'origine asbestosique se développent lentement et ont peu de signification clinique. D'autres réactions pleurales d'origine asbestosique, comme par exemple les pleurésies exsudatives aiguës et les fibroses pleurales progressives, ont une importance clinique. La pathogénie de ces réactions est inconnue, mais une des hypothèses est que des troubles immunologiques seraient en cause. Pour investiguer ceci, nous avons réalisé une caractérisation phénotypique des cellules lymphoïdes dans le sang de 45 patients atteints de lésions pleurales d'origine asbestosique: 20 cas de plaques pleurales (PP), 15 cas de fibrose pleurale diffuse (DPF), et 10 atteints d'épanchement pleural bénin asbestosique (BAPE). Vingt-quatre donneurs de sang bien portants ont servi de contrôles. Tous les groupes asbestosiques réunis avaient un pourcentage de B lymphocytes significativement plus élevé que les contrôles. Le pourcentage de cellules T adjuvantes était significativement plus bas dans les groupes BAPE et DPF que dans les groupes contrôles et PP. Des anomalies significatives des cellules lymphoïdes du sang périphérique existent donc chez les patients atteints de DPF et de BAPE, qui diffèrent des patients atteints de PP, qui eux sont similaires aux normaux.
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